disclosed does not amount to the addition of new matter in violation of 37 CFR § 1.118. *See e.g. Kennecott Corp. v. Kyocera International, Inc.*, 835 F.2d 1419, 5 USPQ2d 1194 (Fed. Cir. 1987).

> By disclosing in a patent application a device that inherently performs a function, operates according to a theory, or has an advantage, a patent applicant necessarily discloses that function, theory, or advantage even though he says nothing concerning it. *Id* at 1422.

It can readily be seen from the amended Table 1, that all the probes have "similar melt" temperatures. Thus, the limitation added to the claim is not new matter.

Moreover, the relationship of primer length and primer "GC" content to annealing temperature is disclosed at page 16, lines 24-34. Specifically the first sentence there states: "The temperature is dependent on the length, the uniqueness of the primer sequence and the relative percentage of GC bases." This passage clearly discloses the importance of length and GC content on annealing temperatures. The fact that all the primers of Table 1 have similar melt temperatures cannot be said to be mere happenstance, in view of the importance of this disclosed relationship.

Furthermore, newly added claims 18-19 do not add new matter either. It can be seen from new Table 1 that, using the most accepted estimation method, the difference between the highest and lowest melting temperature of the probes

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3750 (1986). One skilled in the art readily recognizes that the empirically determined Tms would not be expected to vary significantly, and would also be a simple matter to determine without undue experimentation. The actual ranges of Tms vary only insignificantly among the methods used to estimate them. Thus, the particular method employed is not deemed critical.

is 8.3° C, thus supporting the limitations of claim 18. Claim 19 is similarly supported. It can be seen from new Table 1 that the first primer of sets b. d. and g. and the second primer of sets c. e. and f. have the lower Tms of the pairs. (The primers of set a. both melt at 73° C at 1 M sodium). Using only these lower Tms and calculating the temperature range or variation shows that they all vary by not more than 4.4° C. Thus, not only is claim 1 inherently supported by Table 1 as filed, but so are claims 18 and 19 which all derive from the inherent Tms of the primers disclosed.

Claim 1 has also been amended to remove the requirements of increased times and enzymes. Applicants do not believe this is new matter (as discussed in previous response), however, since these restrictions are not needed to distinguish over the prior art they are removed to facilitate prosecution.

Claim 20 is an amended version of claim 1, wherein it claims the use of multiplex to detect multiple target sequences with or without deletions. This is supported by the specific examples in the specification. In the detection of deletions, the procedure detects the presence or absence of a sequence. If no deletions are present, it detects multiple sequences. Claim 20 corresponds to the situation.

Section 103

In the last office action, the examiner considered Applicants' argument over Kogan et al., but apparently gave no weight to the limitations he viewed as new matter. At least there is no argument of record, pointing out where Kogan, et al. teach that the primer melt temperatures should be balanced. Since

Applicants have shown above that this limitation is not new matter, it should be considered and dealt with by the examiner.

Nowhere in the art is there any suggestion whatsoever that this is an important feature when doing PCR with three or more primer sets. Nowhere in the art of record is there any teaching that the signal from each primer set must "come up" at approximately the same rate. The rate of signal generation is dependent on the efficiency of the extension reaction which, in turn, is dependent on the melt temperature of the primers. However, none of this is derivable from the prior art. Since all primers must be subjected to the same annealing and extension conditions in multiplex PCR, the annealing and extension efficiencies of these primers can vary significantly with melt Tms. Thus, the temperature must be selected based on the primers used. As is well known in the art, the annealing temperature represents a tradeoff or balancing of factors. As lower annealing temperatures are used, the annealing becomes more efficient, but the amplification becomes less specific and the ramp times are longer. Thus, one is pressed into using the highest possible annealing temperature. At any given temperature, the annealing and extension efficiencies of the primers will vary significantly. After 20 cycles of PCR, the variation in the rate of signal generation may result in multifold differences between the signals from the primers depending on their efficiency of annealing at any given temperature. Thus, the signal of one primer pair could go "off the scale," while the signal from another primer pair will barely register, if at all. This is an intolerable situation for multiplex PCR of three or more targets simultaneously.

Since the examiner has cited no prior art that teaches or suggests the criticality of balancing the primer melt temperature characteristics, claim 1 and

claims dependent thereon are not obvious in view of the art. To the contrary, the prior art, (Kogan and Chekob, GG, at least) teach away from the need to balance primer Tms, by using two primer pairs having Tms that vary by at least 15° C and as much as 24° C[2]. In addition, considering only the lower Tm of each pair, the variation is a difference of about 6.6° C. Such a teaching away is a hallmark of nonobviousness and the rejection should be withdrawn.

Additionally, Applicants are including additional references. Please charge the appropriate fee to Account No. 06-2375 from which the undersigned is authorized to draw.

Applicants assert that in view of the above amendments and remarks that the application is now in condition for allowance. Accordingly, Applicants respectfully request that a letters patent be issued on the application as amended. If any requirements remain, please contact the undersigned at (713) 651-5325 for timely resolution.

Respectfully submitted,

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[2]The Tms for Kogan and Chekob were calculated in the same manner as described in new Table 1 and as described in footnote 1 of this response.

Table 1. Summary of DMD gene multiplex amplification primer sets.

	Exon and Size	Primer Sequence	Amplified	Deleted	Tm* ° C
a.	Exon 8 (182bp)	F-GTCCTTTACACACTTTACCTGTTGAG R-GGCCTCATTCTCATGTTCTAATTAG	360 bp	11.3%	73.0 73.0
b.	Exon 17 (178bp)	F-GACTTTCGATGTTGAGATTACTTTCCC R-AAGCTTGAGATGCTCTCACCTTTTCC	416 bp	9.4%	77.4 79.9
c.	Exon 19 (88bp)	F-TTCTACCACATCCCATTTTCTTCCA R-GATGGCAAAAGTGTTGAGAAAAAGTC	459 bp	10.3%	78.1 77.0
d.	4.1Kb Hind III (148bp)	F-CTTGATCCATATGCTTTTACCTGCA R-TCCATCACCCTTCAGAACCTGATCT	268 bp	4.0%	76.9 79.3
e.	0.5Kb Hind III (176bp)	F-AAACATGGAACATCCTTGTGGGGAC R-CATTCCTATTAGATCTGTCGCCCTAC	547 bp	8.4%	81.3 76.3
f.	1.2/3.8Kb Hind III (159bp)	F-TTGAATACATTGGTTAAATCCCAACATG R-CCTGAATAAAGTCTTCCTTACCACAC	506 bp	18.2%	78.8 74.3
g.	Exon 12 (151bp)	F-GATAGTGGGCTTTACTTACATCCTTC R-GAAAGCACGCAACATAAGATACACCT	337 bp	9.6%	73.7 77.4
			Total:	38%	

*Tm = melting temperature, *i.e.*, that temperature at which 50% of strands are dissociated at 1M monovalent cation concentration, calculated by the nearest neighbor method.
~~Values were derived from Td calculated by the Primer Analysis Software Oligo™ (National Biosciences, Plymouth, MN). Values are Tm = Td - 7.6° C.~~
The nearest neighbor method is described by K. J. Breslauer, et al. (1986) *Predicting DNA duplex stability from the base sequence, Proc. Nat. Acad. Sci. USA 83, 3746-3750.* Salt dependence of Tm is described by C. Schildkraut and S. Lifson (1965) *Dependence of the melting temperature of DNA on salt concentration,* Biopolymers 3, 195-208.